Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Polymers with Tunable Side-chain Amphiphilicity as Non-hemolytic Antibacterial Agents

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Materials and instrumentation

All the solvents were of reagent grade and dried prior to use wherever required. Bromoacetyl-bromide, poly(isobutylene-*alt*-maleic anhydride) (Mw~6000 Da, Sigma Catalog no. 531278), 3-aminopropyldimethylamine, 1-Propyl amine, 1-Bromo decane and 1-bromo-2(2-methoxyethoxy)ethane were purchased from Sigma-Aldrich (India) and used as received. 1-Bromo ethane, 1-Bromo butane, 1-Bromo pentane, 1-Bromo heptane and 1-Bromo octane were purchased from Avra chemicals (India) and 1- Propanol and 1-Bromo hexane were obtained from Spectrochem (India) respectively and used as received. Dialysis membrane-150 with a molecular weight cut off of 10 KDa was obtained from HIMEDIA (India). Dialysis tubing, benzoylated with NMWCO of 2 KDa was purchased from Sigma-Aldrich (India). NMR spectra were recorded using Bruker AMX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer. The chemical shifts (δ) are reported in parts per million downfield from the peak for the internal standard TMS for ^{1}H NMR and ^{13}CNMR. Infrared (IR) spectra of the solid compounds were recorded on Bruker IFS66 V/s spectrometer using KBr pellets. IR spectra of the compounds soluble in low-boiling solvents were recorded with the same instrument using NaCl crystal. Optical density and absorbance were measured by Tecan InfinitePro series M200 Microplate Reader. Bacterial strains, *P. aeruginosa* (MTCC 424), *S. aureus* (MTCC 737) and *E. coli* (MTCC 443) were purchased from MTCC (Chandigarh, India). *E. faecium* (ATCC 19634), β-lactamase producing and drug-resistant *K. pneumoniae* (ATCC 700603), methicillin resistant *S. aureus* (MRSA) (ATCC 33591), vancomycin resistant *E. faecium* (VRE) ((OrlaJensen) Schleifer and Kilpper-Balz, ATCC 51559) were obtained from ATCC (Rockville, Md).

Microorganisms and culture conditions

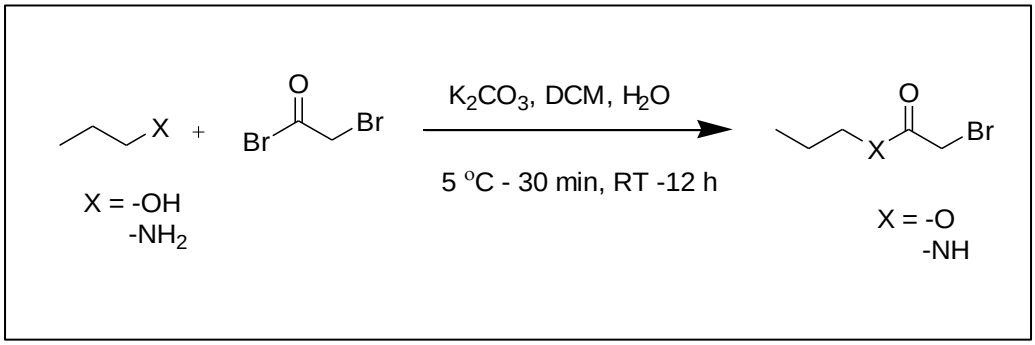
The antibacterial activity of the polymers was done against both Gram-negative (*E. coli, P. aeruginosa* and *K. pneumoniae)* and Gram-positive (*S. aureus* and *E. faecium*) bacteria including the drug resistant strains VRE and MRSA. *E. coli* was cultured in Luria Bertani broth (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl in 1000 mL of sterile distilled water (pH -7) while *S. aureus, P. aeruginosa* and MRSA were grown in Yeast-dextrose broth (1 g of beef extract, 2 g of yeast extract, 5 g of peptone and 5 g of NaCl in 1000 mL of sterile distilled water). Both *E. faecium* and VRE were cultured in Brain Heart Infusion broth (BHI). *K. pneumoniae* was grown in nutrient media (3 g of beef extract and 5 g of peptone in 1000 mL of sterile distilled water). For solid media, 5% agar was used along with above mentioned composition. The bacterial samples were freeze dried and stored at -80°C. 5 μL of these stocks were added to 3 mL of the nutrient broth and the culture was grown for 6 h at 37 °C prior to the experiments.

I. Synthetic Procedures and Characterization of all the Polymeric Derivatives

i) Synthesis of Amide or Ester Based Alkylating Agents[1]

N-propyl-1-bromoethanamide: Propylamine (7 g, 118 mmol) was dissolved in dichloromethane (55 mL). Potassium carbonate, K_2CO_3 (24.55 g, 178 mmol) was dissolved in 60 mL of distilled water and the solution was added to the organic solution. The resulting two phase solution was cooled to 5 °C. A solution of bromoacetyl bromide (35.85 g, 178 mmol) in dichloromethane (55 mL) was carefully added drop wise to the cooled solution while maintaining the temperature at 5 °C for about 30 min. Then the reaction mixture was stirred at room temperature for 12 h. The aqueous solution was separated and washed with dichloromethane (2 × 25 mL). The organic solution was washed with water (2 × 50 mL) and passed over the anhydrous Na_2SO_4 and

concentrated to yield an oily liquid quantitatively: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, $CDCl_3$): δ/ppm 0.878 (t, terminal –*CH₃*, 3H), 1.543 (m, -*CH₂*CH_3-, 2H), 3.278 (t, -CONH*CH₂*-, 2H), 3.881 (s, -CO*CH₂*Br, 2H), 6.475 (br s, amide –*NH*CO, 1H)); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.195, 22.768, 26.904, 29.324, 29.423, 29.588, 29.646, 29.708, 31.995, 40.403, 165.589.



Scheme S1 General synthetic route for the synthesis of ester and amide based alkylating agents

Propyl-1-bromoethanoate: 1-Propanol (7 g, 116.5 mmol) was dissolved in dichloromethane (55 mL). Potassium carbonate, K_2CO_3 (19.32 g, 140 mmol) was dissolved in 60 mL of distilled water and the solution was added to the organic solution. The resulting two phase solution was cooled to 5 °C. A solution of bromoacetyl bromide (28.21 g, 140 mmol) in dichloromethane (55 mL) was carefully added drop wise to the cooled solution while maintaining the temperature at 5 °C for about 30 min. Then the reaction mixture was stirred at room temperature for 12 h. The aqueous solution was separated and washed with dichloromethane (2 × 25 mL). The organic solution was washed with water (2 × 50 mL) and passed over the anhydrous Na_2SO_4 and concentrated to yield an oily liquid quantitatively: FT-IR: 2950-2850 (C-H str.), 1735 cm^{-1} (C=O str.), 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, $CDCl_3$): δ/ppm 0.85

(t, terminal –*CH*$_3$, 3H), 1.57 (m, -*CH*$_2$CH$_3$-, 2H), 4.0 (t, -COO*CH*$_2$-, 2H), 3.7 (s, -CO*CH*$_2$Br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.195, 22.768, 26.904, 29.324, 29.423, 29.588, 29.646, 29.708, 31.995, 40.403, 171.19.

ii) Synthesis of Polymeric Derivatives

Poly(isobutylene-alt-N-(N', N' -dimethylaminopropyl)-maleimide);PIBMI:

To a solution of 10 g of poly(isobutylene-*alt*-maleic anhydride) (PIBMA) (Avg. Mw = 6000 g/mol) in 60 ml of DMF, 7.96 g of 3-Aminopropyldimethylamine (1.2 Equi. or 78 mmol with respect to the monomer weight of the polymer (154 g/mol)) was added and stirred at 120 °C for 48 h in a screw-top pressure tube. The reaction mixture was cooled, precipitated with 200 mL of distilled water and was centrifuged at 10,000 rpm for 15 min. The polymer was dried at 45 °C for 24 h under vacuum to give a pale yellow solid with 100% yield (complete conversion of the anhydride to imide was confirmed by complete disappearance of peaks at 1850 cm^{-1} (C=O asym. str.) and 1785 (C=O sym. str.) for the anhydride ring and appearance of peaks 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) for the imide ring by FT-IR). FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.), 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, $CDCl_3$): δ/ppm 0.7–1.2 (br $CH_2C(CH_3)_2$, 6H), 1.7 (br $CH_2C(CH_3)_2$, 2H), 1.86 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.2-2.5 (br $NCH_2CH_2CH_2N(CH_3)_2$, 8H), 2.7–3.1 (br, *CHCH*, 2H), 3.6 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 179.9, 179.7, 179.4, 177.4, 177.3, 177.2, 55.5, 45.9, 45.5, 44.1, 40.8, 40.6, 40.2, 40.0, 37.4, 26.2, 25.5, 24.8, 24.7, and 24.6.

Protonated PIBMI derivative, QPro_PIBMI: 0.5 g of PIBMI was dissolved in 10 mL of 2 M HBr solution and stirred at room temperature for 12 h. The product was obtained by dialysing against DI water (benzoylated dialysis tubing with NMWCO of 2 KDa) at 4 °C followed by freeze-drying with 100% yield. FT-IR: 3300 cm^{-1} (N-H str.), 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.7–1.2 (br $CH_2C(CH_3)_2$, 6H), 1.7 (br $CH_2C(CH_3)_2$, 2H), 2.0 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.8-2.9 (br $NCH_2CH_2CH_2N(CH_3)_2$, 8H), 2.7-3.1 (br, *CHCH*, 2H), 3.6 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H).

Poly (isobutylene-alt-N-(N', N'–dimethyl N'-(n)-alkyl aminopropyl)-maleimide) – QAlk_PIBMI: To a solution of 0.5 g of PIBMI in 20 mL of DMF/$CHCl_3$ (1:1), 1.04 g of 1-bromoalkane (n = 2, 4, 5, 6, 7, 8 and 10)/ 1.15 g of 1-bromo-2(2-methoxyethoxy) ethane (3 Equi. or 6.3 mmol with respect to the monomer weight of PIBMI (238.18 g/mol)) was added and stirred at 75 °C for 96 h in a screw top pressure tube. The solution was cooled, precipitated with 40 mL of n-hexane/diethylether and filtered. The white solid was washed with n-hexane (4 × 40 mL)/diethylether and dried at 40 °C for 12 h under vacuum (yield: 100%).

QDec_PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.85-0.9 (br terminal $–CH_3$, 3H), 0.95–1.2 (br $CH_2C(CH_3)_2$, 6H), 1.3-1.5 (br $CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2$, 16H) 1.7 (br $CH_2C(CH_3)_2$, 2H), 2.0 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br *CHCH*, 2H), 3.1-3.3 (br $NCH_2CH_2CH_2NCH_2(CH_3)_2$, 10H), 3.6 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H).

QOct_PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.85-0.9 (br terminal –*CH₃*, 3H), 0.95–1.2 (br $CH_2C(CH_3)_2$, 6H), 1.3-1.5 (br $CH_2CH_2CH_2CH_2CH_2CH_2$, 12H) 1.7 (br $CH_2C(CH_3)_2$, 2H), 2.0 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br *CHCH*, 2H), 3.1-3.3 (br $NCH_2CH_2CH_2NCH_2(CH_3)_2$, 10H), 3.6 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H).

QHep_PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.85-0.9 (br terminal –*CH₃*, 3H), 0.95–1.2 (br $CH_2C(CH_3)_2$, 6H), 1.3-1.5 (br $CH_2CH_2CH_2CH_2CH_2$, 10H) 1.7 (br $CH_2C(CH_3)_2$, 2H), 2.0 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br *CHCH*, 2H), 3.1-3.3 (br $NCH_2CH_2CH_2NCH_2(CH_3)_2$, 10H), 3.6 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H).

QHex_PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.85-0.9 (br terminal –*CH₃*, 3H), 0.95–1.2 (br $CH_2C(CH_3)_2$, 6H), 1.3-1.5 (br $CH_2CH_2CH_2CH_2$, 8H) 1.7 (br $CH_2C(CH_3)_2$, 2H), 2.0 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br *CHCH*, 2H), 3.1-3.3 (br $NCH_2CH_2CH_2NCH_2(CH_3)_2$, 10H), 3.6 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H).

QPen_PIBMI: - FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.85-0.9 (br terminal –*CH₃*, 3H), 0.95–1.2 (br $CH_2C(CH_3)_2$, 6H), 1.3-1.5 (br $CH_2CH_2CH_2$, 6H) 1.7 (br $CH_2C(CH_3)_2$, 2H), 2.0 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br *CHCH*, 2H), 3.1-3.3 (br $NCH_2CH_2CH_2NCH_2(CH_3)_2$, 10H), 3.6 (br $NCH_2CH_2CH_2N(CH_3)_2$, 2H).

QBut_PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.85-0.9 (br terminal –*CH₃*, 3H), 0.95–1.2 (br $CH_2C(CH_3)_2$, 6H), 1.3-1.5 (br CH_2CH_2, 4H) 1.7 (br

CH$_2$C(CH$_3$)$_2$, 2H), 2.0 (br NCH$_2$*CH*$_2$CH$_2$N(CH$_3$)$_2$, 2H), 2.7–3.1 (br *CHCH*, 2H), 3.1-3.3 (br NCH$_2$CH$_2$*CH*$_2$N*CH*$_2$*(CH*$_3$*)*$_2$, 10H), 3.6 (br N*CH*$_2$CH$_2$CH$_2$N(CH$_3$)$_2$, 2H).

QEth_PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D$_2$O): δ/ppm 0.85-0.9 (br terminal –*CH*$_3$, 3H), 0.95–1.2 (br CH$_2$C*(CH*$_3$*)*$_2$, 6H), 1.7 (br *CH*$_2$C(CH$_3$)$_2$, 2H), 2.0 (br NCH$_2$*CH*$_2$CH$_2$N(CH$_3$)$_2$, 2H), 2.7–3.1 (br *CHCH*, 2H), 3.1-3.3 (br NCH$_2$CH$_2$*CH*$_2$N*CH*$_2$*(CH*$_3$*)*$_2$, 10H), 3.6 (br N*CH*$_2$CH$_2$CH$_2$N(CH$_3$)$_2$, 2H).

QOEG_PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D$_2$O): δ/ppm 0.95–1.2 (br CH$_2$C*(CH*$_3$*)*$_2$, 6H), 1.7 (br *CH*$_2$C(CH$_3$)$_2$, 2H), 2.0 (br NCH$_2$*CH*$_2$CH$_2$N(CH$_3$)$_2$, 2H), 2.7–3.1 (br *CHCH*, 2H), 3.1-3.2 (br NCH$_2$*(CH*$_3$*)*$_2$, 6H), 3.45 (s, terminal –*CH*$_3$), 3.55-3.8 (br, N*CH*$_2$CH$_2$*CH*$_2$N*CH*$_2$(CH$_3$)$_2$ and O*CH*$_2$*CH*$_2$O, 10H), 4.0 (br O*CH2*CH$_2$N(CH$_3$)$_2$, 2H).

Poly(isobutylene-alt-N-(N', N' –dimethyl N'-(propyl ethanoate/N'' –propyl ethanamide) aminopropyl)-maleimide) – QEst_PIBMI/ QAmi_PIBMI: To a solution of 0.5 g of PIBMI in 20 mL of dry DMF/dry CHCl$_3$ (1:1), 0.76 g of propyl-1-bromoethanoate/ N-propyl-1-bromoethanamide (2 Equi. or 4.2 mmol with respect to the monomer weight of PIBMI (238.18 g/mol)) was added and stirred at 65°C-75°C for 96 h in a screw top pressure tube. The solution was cooled, precipitated with 40 mL of diethylether and filtered. The white solid was washed with diethylether (4 × 40 mL) and dried at 40 °C for 4 h under vacuum (yield: 100%).

QEst_PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.), 1735 cm^{-1} (ester C=O str.) 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O

str.); ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.85 (br, terminal –*CH₃*, 3H), 0.95–1.2 (br, $CH_2C(CH_3)_2$, 6H), 1.57 (br,-$COOCH_2CH_2CH_3$, 2H), 1.7 (br, $CH_2C(CH_3)_2$, 2H), 2.0 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.1-3.3 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 8H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 3.7 (br, -$N(CH_3)_2COCH_2$, 2H) 4.0 (br, -$COOCH_2$-, 2H).

QAmi_PIBMI: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.878 (br, terminal –*CH₃*, 3H), 0.95–1.2 (br, $CH_2C(CH_3)_2$, 6H), 1.543 (br, -$CONHCH_2CH_2CH_3$-, 2H), 1.7 (br, $CH_2C(CH_3)_2$, 2H), 2.0 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.1-3.3 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 8H), 3.5 (br, -$CONHCH_2$-, 2H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 3.8 (br, -$N(CH_3)_2COCH_2$, 2H).

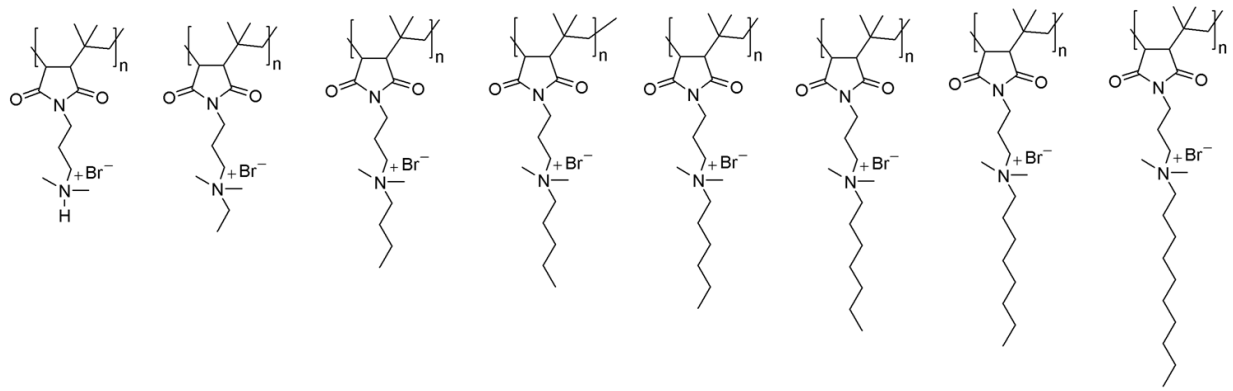


Fig. S1 Structures of QPro_PIBMI and QAlk_PIBMI derivatives with n-alkyl side chains

*iii)**Chemical Degradation of QEst_PIBMI/QAmi_PIBMI/QPen_PIBMI-***

The hydrolysis of the QEst_PIBMI/QAmi_PIBMI was done using 8 M HCl at 50 °C for 72 h to give the zwitterionic derivative QZwi_PIBMI. Treatment of either the QEst_PIBMI or

QZwi_PIBMI with 1 M NaOH at 50 °C for 24 h degraded the succinimide ring yielding the corresponding open ring by-product with net anionic charge. Similarly, the succinimide ring opening of QPen_PIBMI was achieved after heating in 1 M NaOH at 50 °C for 24 h. All these by-products were obtained after dialyzing against DI water at room temperature using a dialysis membrane (Mol. wt. cut off =10 KDa) followed by freeze-drying. Poly(isobutylene-*alt*-maleic acid) was synthesized by treatment of poly(isobutylene-*alt*-maleic anhydride) with 1 M NaOH at 80 °C for 24 h followed by dialysis against DI water (benzoylated dialysis tubing with NMWCO of 2 KDa) at 4 °C and freeze-drying. With respect to all the derivatives, the complete conversion from the reactant to the product was confirmed quantitatively by FT-IR.

QZwi_PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1634 cm^{-1} (carboxylate C=O str.), (1470-1410 cm^{-1} (C-C str.), 1290-1110 (C-O str.) ; ^{1}HNMR (400 MHz, D_2O): δ/ppm 0.95–1.2 (br, $CH_2C(CH_3)_2$, 6H), 1.7 (br, $CH_2C(CH_3)_2$, 2H), 2.0 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.1-3.3 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 8H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 4.1-4.3 (br, $-N(CH_3)_2COCH_2$, 2H).

QOpr_PIBMI: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 (C-H str.), 1680 cm^{-1} (amide I, C=O str.), 1634 cm^{-1} (zwitterionic carboxylate C=O str.), 1580 cm^{-1} (sodium carboxylate C=O str), 1560 cm^{-1} (Amide II, N-H ben.), (1470-1410 cm^{-1} (C-C str.), 1290-1110 (C-O str.).

PIBMA: FT-IR: 2950-2850 (C-H str.), 1720 cm^{-1} (C=O str.), (1470-1410 cm^{-1} (C-C str.), 1290-1110 (C-O str.).

QOPen_PIBMI: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 (C-H str.), 1680 cm^{-1} (amide I, C=O str.), 1580 cm^{-1} (sodium carboxylate C=O str), 1560 cm^{-1} (Amide II, N-H ben.), (1470-1410 cm^{-1} (C-C str.), 1290-1110 (C-O str.).

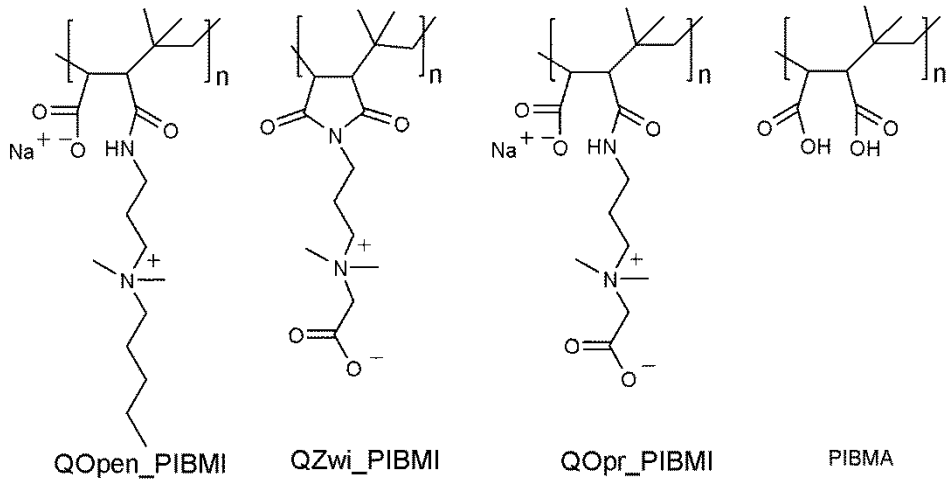
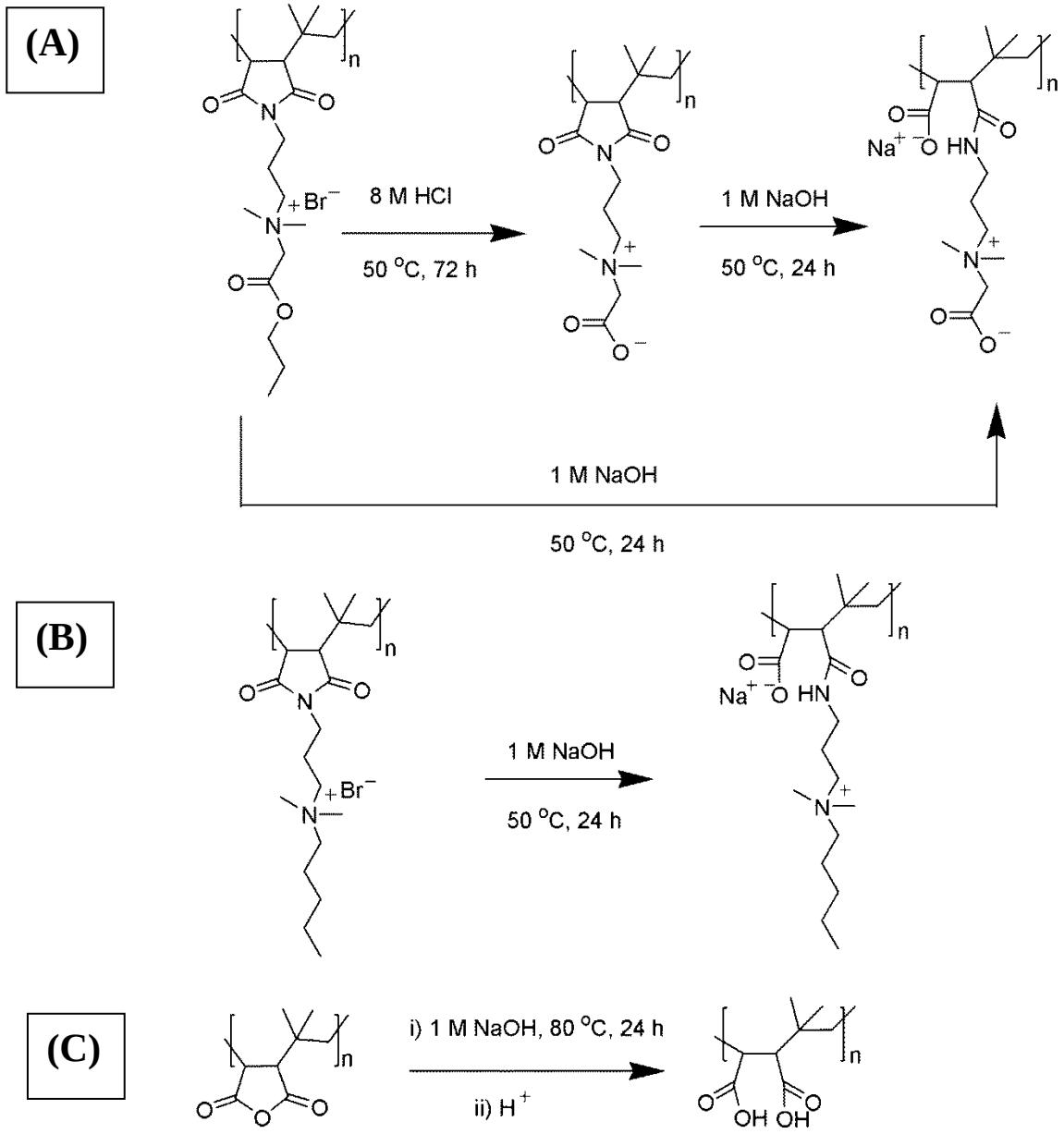


Fig. S2 Structures of degraded polymeric by-products of QEst/Ami/Pen_PIBMI derivatives



Scheme S2 Synthesis of degradation by-products of QEst_PIBMI/QAmi_PIBMI and QPen_PIBMI

iv) Degree of Quaternization:

Degree of quaternization of the polymeric derivatives was calculated using ^{1}H NMR analysis following a literature procedure [2-4].

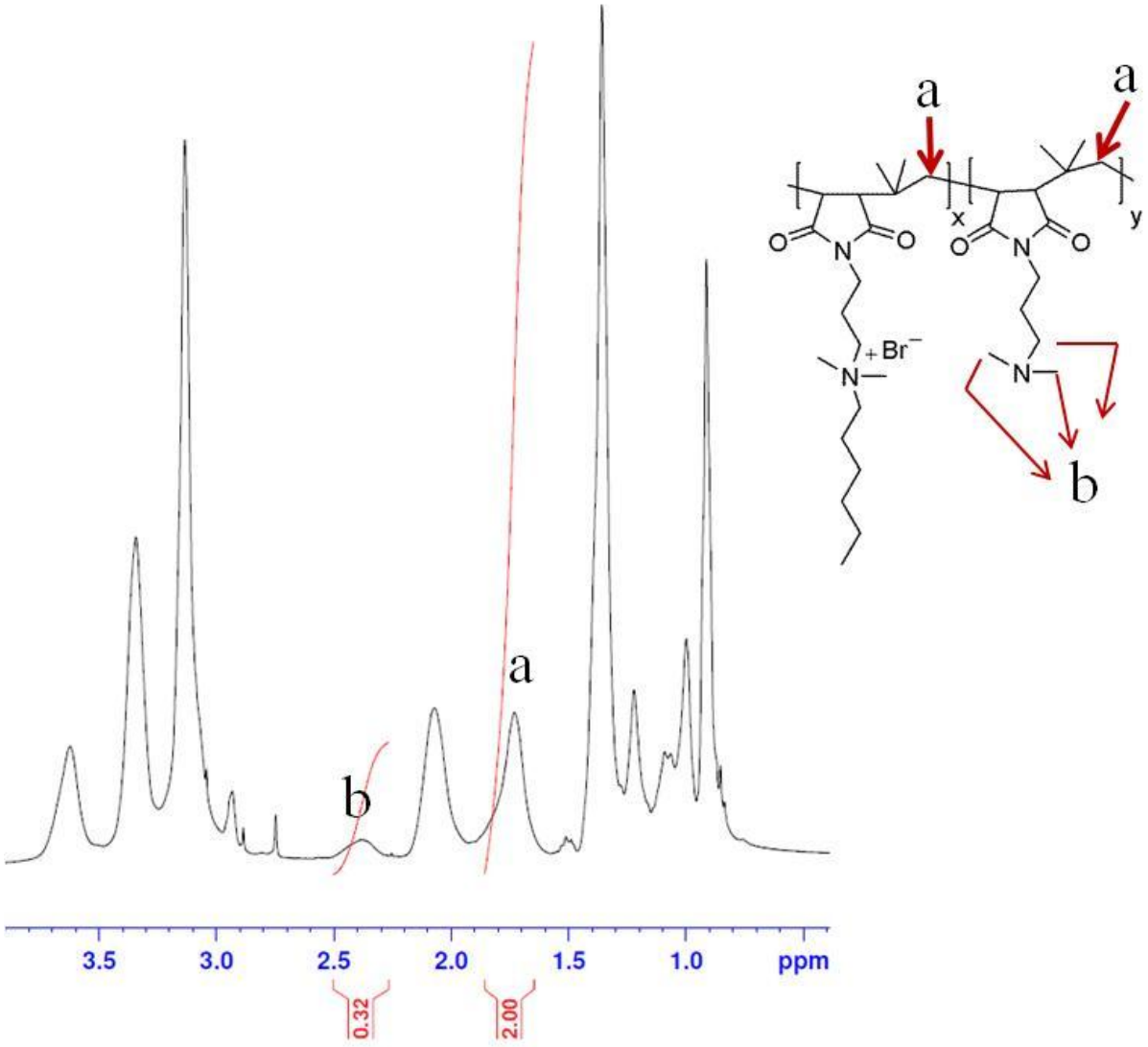


Fig. S3 ^{1}H NMR of QHex_PIBMI (in D_2O) indicating the peaks used for the calculation of degree of quaternization (δ/ppm: (a) for 1.7 (br ***CH₂***C$(CH_3)_2$, 2H) and (b) for 2.2-2.5 (br, NCH_2CH_2***CH₂N(CH₃)₂***, 8H)

Degree of quaternization (x) = (1- y) × 100 %

Wherein $y = \{([\boldsymbol{CH_2}N\boldsymbol{(CH_3)_2}]/8)/ ([\boldsymbol{CH_2}C(CH_3)_2]/2)\}$

$y = \{(m/8)/ (n/2)\}$, $m = [\boldsymbol{CH_2}N\boldsymbol{(CH_3)_2}]$ and $n = [\boldsymbol{CH_2}C(CH_3)_2]$

For e.g. QHex_PIBMI, Degree of quaternization (x) = $1-\{(0.32/8) / (2.0/2)\} \times 100$ %

= 96 %

Wherein, $[\boldsymbol{CH_2}C(CH_3)_2]$ and $[\boldsymbol{CH_2}N\boldsymbol{(CH_3)_2}]$ are the integrals of the hydrogens (a and b respectively, shown in Fig. S3) those are bold and italicized.

The molecular weight (number average molecular weight, M_n) (Fig. S3) of the final derivatives is calculated based on the molecular weight of the precursor (average M_w~ 6000 Da, monomer weight is 154 g/mol and n~39) and the degree of quaternization[2-4].

Table S1: Degree of Quaternization and Molecular Weight of the Polymeric Derivatives

Polymer	Degree of Quaternization ^{1}H NMR/%	M_n (10^4 g/mol)[a]
QEth_PIBMI	95	1.63
QBut_PIBMI	95	1.73
QPen_PIBMI	96	1.79
QHex_PIBMI	96	1.85
QHep_PIBMI	94	1.88
QOct_PIBMI	93	1.92
QDec_PIBMI	94	2.0
QAmi_PIBMI	98	1.92
QEst_PIBMI	96	1.90
QOEG_PIBMI	97	1.92

[a] Calculated from molecular weight of precursor copolymers and degree of quaternization.

Bio-assays of the Polymeric Derivatives:

Antibacterial Assays

Antibacterial activity was determined with the slight modifications of standardized protocols published by Weigand *et al*[5]. Water-soluble QAlk_PIBMI derivatives were assayed in a modified micro-dilution broth format[1]. Stock solutions of the quaternized PIBMI derivatives were made by serially diluting the compounds using autoclaved Millipore water. Bacteria, to be tested, grown for 6 h in the suitable media contained ~10^9 CFU mL^{-1} (determined by spread plating method), which was then diluted to 10^5 CFU mL^{-1} using nutrient media. 50 μL of serially diluted compound was added to a 96 well plate (Polystyrene) containing 150 μL bacterial solutions. Two controls were made; one containing 150 μL of media and 50 μL of compound and the other containing 150 μL of bacterial solution and 50 μL water . The plate was then incubated at 37 °C for a period of 24 h and the O.D. value was measured at 600 nm using a Tecan InfinitePro series M200 Microplate Reader. MIC value was determined by taking the average of triplicate O.D. values for each concentration and plotting it against concentration using Origin Pro 8.0 software. The data was then subjected to sigmoidal fitting. From the curve the MIC value was determined, as the point in the curve where the O.D. was similar to that of control having no bacteria. The MIC values and errors are reported as averages and standard errors of mean of three independent experiments respectively. MIC curves for each polymer are representative data from the three independent experiments and each experiment was performed in triplicates.

Hemolytic Assays

The hemolytic activity was determined against human erythrocytes with slight modifications to our previously published literature[1]. Erythrocytes were isolated from freshly drawn, heparanized human blood and resuspended to 5 % v/v in PBS (pH 7.4). In a 96-well microtiter plate, 150 µL of erythrocyte suspension was added followed by 50 µL of serially diluted compound to give a final solution of 3.75 % v/v erythrocytes. PBS buffer was added instead of polymer solution as negative hemolysis control and Triton X-100 (1% v/v) was used as positive hemolysis control. The plate was incubated for 1 h at 37°C and was then centrifuged at 3,500 rpm for 5 min. 100 µL of the supernatant was then transferred to a fresh micro titer plate and absorbance at 540 nm was measured using a Tecan InfinitePro series M200 Microplate Reader. Percentage of hemolysis was determined as $(A - A_0)/ (A_{total} - A_0) \times 100$, where A is the absorbance of the test well, A_0 the absorbance of the negative controls, and A_{total} the absorbance of 100% hemolysis wells, all at 540 nm. Hemolysis was plotted as a function of polymer concentration and the HC_{50} was defined as the polymer concentration, which causes 50% hemolysis relative to the positive control. In some cases, hemolysis did not reach 50 % up to the highest polymer concentration tested and the HC_{50} was not determined. The HC_{50} values and errors are reported as averages and standard errors of mean of three independent experiments, respectively. Hemolysis curves for each polymer are representative data from two independent experiments and each experiment was performed in triplicates.

Bactericidal Time-kill Kinetics

The bactericidal activity of the derivatives was assessed by the kinetics or the rate at which it affects the killing action of the compound. Briefly, *S. aureus* was grown in yeast-dextrose broth

at 37 °C for 6 h. Test compound, QAmi_PIBMI, having the final concentrations of 1×MIC, 6×MIC and 12×MIC was inoculated with the aliquots of *S. aureus* resuspended in fresh media at approximately 1.8×10^5 CFU mL^{-1}. After specified time intervals (0, 1, 2 and 3 h), 20 μL aliquots were serially diluted 10 fold in 0.9 % saline, plated on sterile yeast-dextrose agar plates and incubated at 37 °C overnight. The viable colonies were counted the next day and represented as $\log_{10}$ (CFU mL^{-1}).

Antibaterial Efficacy in Human Plasma

The antibacterial activity of the derivatives was performed in presence of 50% of plasma to assess its susceptibility to plasma proteases[6]. 250 μL of the compound was added to 250 μL of human plasma (centrifuged from whole blood and collected the blood minus cell fraction) and preincubated at 37 °C for 0 and 3h (final concentration of human plasma is 50% (vol/vol)). After incubation, the compound was diluted two-fold in 0.9% saline, performed the antibacterial assay against *S. aureus* and MIC was determined as described above. Also, a similar MIC experiment against *S. aureus* was performed in the absence of the plasma as control.

Cytoplasmic Membrane Depolarization Assay[7,8]

Mid-log phase bacterial cells were harvested, washed with 5 mM HEPES and 5 mM glucose and resuspended in 5 mM glucose, 5 mM HEPES buffer and 100 mM KCl solution in 1:1:1 ratio (~10^9 CFU mL^{-1}). Measurements were made in a cuvette containing 2 mL of bacterial suspension and 2 μM di$SC_3$5. The fluorescence of the dye was monitored for 10 min (*S. aureus*) to 20 min (*E. coli*) at RT using PerkinElmer LS-55 Luminescence Spectrometer at excitation wavelength of 622 nm and emission wavelength of 670 nm. Dye uptake, and resultant self

quenching, was modulated by the membrane potential. After reaching the maximum uptake of the dye by bacteria, which was indicated by a minimum in dye fluorescence, (after 10 min for *S. aureus* and 20 min for *E. coli*) quaternized PIBMI derivatives (50 μg mL^{-1}) were added to the cells, and the decrease in potential was monitored by the increase in fluorescence for further 10 min.

Cytoplasmic Membrane Permeabilization Assay [9]

Mid-log phase (grown for 6 h) *E. coli and S. aureus* cells were harvested (4000 rpm, 4°C, 10 min), washed, and resuspended in PBS buffer of pH 7.2. Then quaternized PIBMI derivatives were added (50 μg mL^{-1}) to the cuvette containing 2.0 mL of bacterial suspension and 10 μM propidium iodide (PI). Excitation wavelength of 535 nm (slit width: 10 nm) and emission wavelength of 617 nm (slit width: 10 nm) were used. The uptake of PI was measured by the increase in fluorescence of PI for 10 min as a measure of membrane permeabilization.

Morphological Membrane Disruption by FESEM Analysis [1]

Bacteria were cultured for 6 h in suitable media (Yeast-dextrose broth for *S. aureus* and LB broth for *E. coli*) at 37°C. The cells were centrifuged and resuspended in respective nutrient media at pH 7.4 (10^8 CFU mL^{-1}). The suspension was divided into two portions (1 mL each). To one portion was added a solution of QAmi_PIBMI (6×MIC). The other portion was used as a control and left untreated. The suspensions were then incubated at 37 °C for 2 h (at ~ 250 rpm shaking speed), and the bacteria from both tubes were harvested by centrifugation at 12000 rpm for 1 min. Finally, the cells were sequentially dehydrated with 30, 50, 70, 80, 90, and 100% ethanol. 5 μL of dehydrated cells was then dropped on a small piece of silicon wafer and dried at room

temperature. Before being imaged, the silicon wafers containing bacteria were sputter coated with gold. Images were recorded by using Quanta 3D FEG FEI field-emission scanning electron microscopy at 5 kV or 8 kV operating voltage.

II. Supplementary Tables and Figures

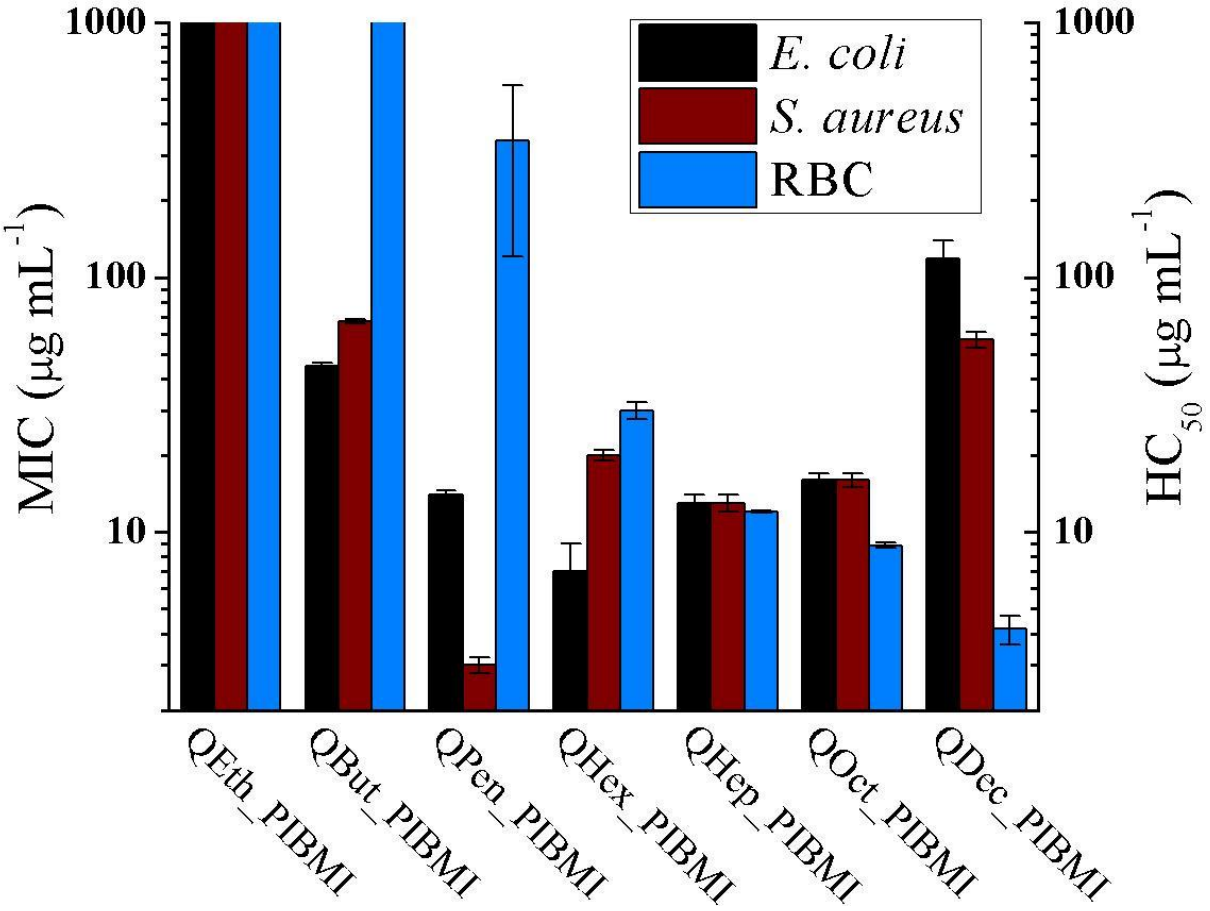


Fig. S4 Antibacterial activities of the QAlk-PIBMI (C_2-C_{10}) against *E. coli* and *S. aureus* showing the parabolic relationship and hemolytic activities showing the decreasing order with increase of hydrophobicity.

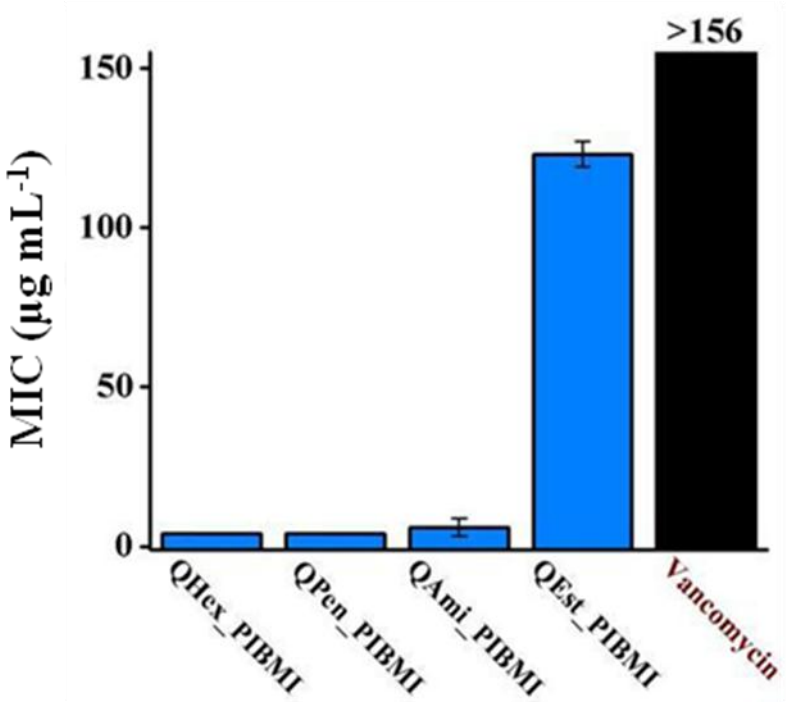


Fig. S5 The antibacterial activity of the derivatives against VRE

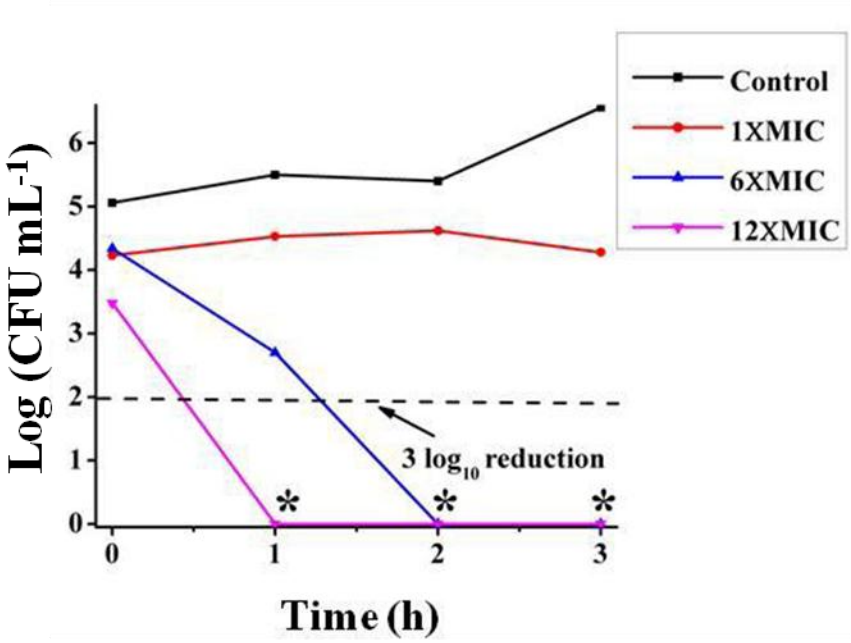


Fig. S6 Bactericidal time-kill kinetics of QAmi_PIBMI against *S. aureus* (stars represent <50 CFU mL^{-1}).

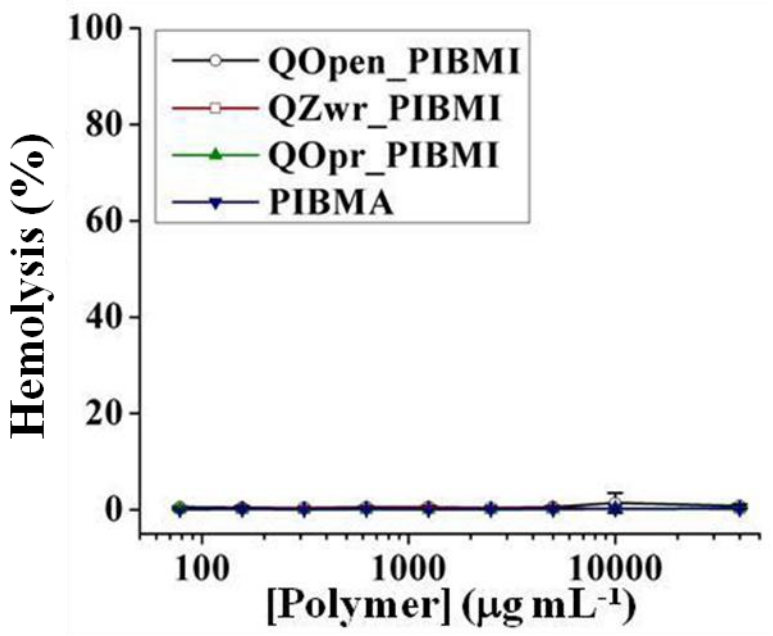


Fig. S7 The hemolytic activity of the degraded polymeric by-products

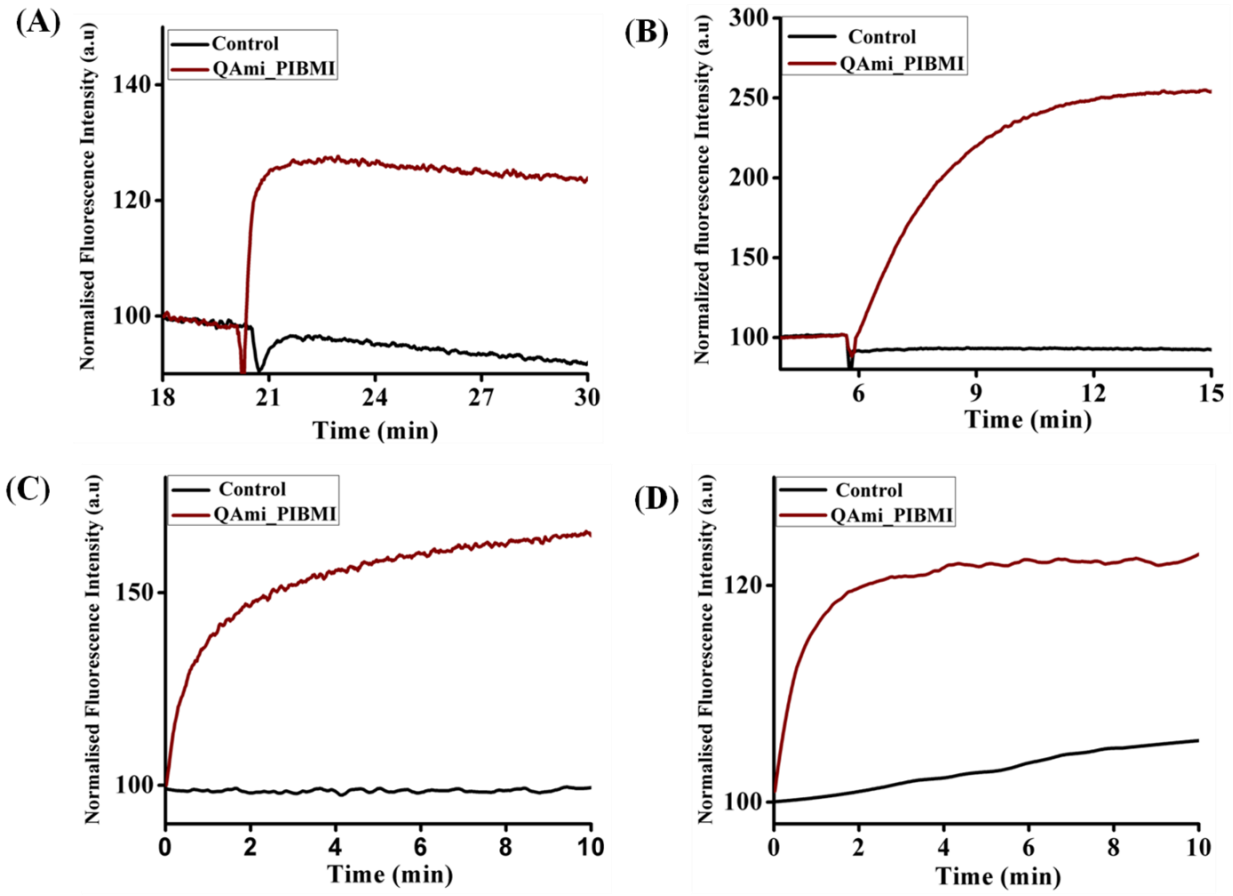


Fig. S8 Membrane-active properties of the quaternized polymeric derivative, QAmi_PIBMI. Cytoplasmic membrane depolarization of the *E. coli* (A) and *S. aureus* (B) and cytoplasmic membrane permeabilization of *E. coli* (C) and *S. aureus* (D) treated with different quaternized polymeric derivatives. Similar to antimicrobial peptides, these polymeric mimics also depolarize and permeabilize the bacterial membranes.

III. Supplementary References

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